# MICROSTIMULATION OF LUMBOSACRAL SPINAL CORD-MAPPING

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**Eighth Progress Report**
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**Neural Prosthesis Program**

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## I. Introduction

During this quarter progress was made in three areas of investigation: (1.) The use of two electrodes located in two different spinal cord segments which control antagonistic muscles (i.e. the knee joint flexors and extensors). Co-activation of these groups of muscles would produce limb stiffness with ability to position and hold the hindlimb at a given position. Our preliminary results show that equal torque generated in antagonistic muscles can result in a net torque of zero and therefore should maintain the limb at a given position. Since shank movement is non-linear and many dynamic and static factors are involved in moving to and maintaining a given limb position, a positional feedback system seems necessary to produce smooth limb movements with accurate positioning. (2.) During this quarter we continued our tracing studies with pseudorabies virus (PRV) to determine the location and distribution of neurons and interneurons which control the external urethral sphincter (EUS). These studies have identified at least three groups of interneurons as well as establishing the location of the first order efferent neurons in Onuf's nucleus which project to the striated muscles of the EUS. (3.) During this quarter tracing studies were begun using the transsynaptic tracer PRV, to determine the location of preganglionic and interneurons which project to the prostate gland. Like most urogenital organs the prostate receives input from both the sympathetic (lumbar) and parasympathetic (sacral) components of the autonomic nervous system. Numerous interneurons at both the lumbar and sacral levels of the spinal cord as well as preganglionic neurons have been identified. These sites will be targets for future microstimulation experiments.

## II. Microstimulation at Two Spinal Cord Sites to Produce Activation of Antagonistic Flexor and Extensor Muscle Groups.

These studies, begun during this quarter, examined the changes in torque about the knee joint to individual and co-activation of flexor and extensor muscle groups. These experiments provide information concerning the interactions that occur when flexor and extensor muscles are activated simultaneously. Such interaction could occur when making a smooth transition in limb position or maintaining a specified joint angle, which would occur, for example, in walking and standing.

The experimental setup is similar to that described in detail in previous progress reports and briefly described below, together with a few specific changes for these particular experiments. Cats are anesthetized with pentobarbital iv (25 - 30 mg/kg) and fixed in a spinal frame during the entire experiment. A rotational torque sensor is attached to the tibia via a bar with the pivot point at the knee joint (see Figure 1). Torque about the knee joint and EMG from various flexor and extensor muscles of the hindlimb are displayed and recorded ( Fig. 1). A laminectomy exposes the spinal cord and roots from $S_3$ to $L_4$. In these experiments, two fine tipped (300 - 400 $u^2$ exposed tip) activated iridium microelectrodes are positioned, one in $L_6$, to produce a maximum extension torque and EMG, and a second electrode is positioned in either $L_7$ or $S_1$ to produce a maximum flexion torque and EMG. The stimulus parameters for each electrode is 40 Hz, 0.2 msec duration, 12 seconds on and 120 seconds off. Each electrode is first tested individually at intensities from 10 to 100 uA, then both electrodes are activated together with each combination of intensities from 10 to 100 uA. Figure 2 shows torque and EMG data from one series of experiments where maximum flexion was first produced to a 100uA stimulus in the $S_1$ spinal segment, 3.0 mm from the cord surface (Figure 2A). In figure 2A only flexor muscles are activated and only flexor torque and EMG are recorded. Extension torque was then produced with increasing intensities of stimulation (Figure 2B - F) of the $L_6$ cord at a location in the lateral ventral horn 3.4 mm from the spinal cord surface. As the extension torque became greater with each increase in stimulus intensity the flexion torque was off-set until, in Figure 2C, the extension was just slightly greater and a net extension torque was recorded. As the net extension torque increased with increasing intensity of stimulation the extension EMG activity likewise increased. It should be noted that the maximum flexion torque recorded at all sites in both $L_7$ and $S_1$ were always less than the maximum extension torque elicited from $L_6$ and $L_5$ stimulation. This is probably due to the fact that the extensor muscles (mainly the quadriceps) are larger, more powerful muscles than the flexors (biceps femoris and semitendinosus). The maximum torque generated by the flexor are, in general, one-half to one-third the torque produced by the extensors.

Figure 3 is a three dimensional surface plot showing data for peak net torque generated by pairing all combinations of each of seven (0, 10, 20, 40, 60, 80, 100 uA) intensities of stimulation to each of two electrodes which produce either flexion ($S_1$ or $L_7$) or extension ($L_6$). The plot

generated indicates that co-activation of flexors and extensors at specific intensities of stimulation can maintain net torque at zero. This suggesting that at these combinations of intensities the limb could be held at a given position with a constant amount of stiffness. As the intensity of stimulation is increased, for example, to the extensor site ($L_6$) or decreased to the flexor site ($L_7$ or $S_1$) the net torque would increase in the direction of extension and the limb should change position to an angle of greater extension. By controlling two sites in the spinal cord with microstimulation, smooth limb movement and maintenance of limb position could be accomplished with optimal selection of stimulus parameters. Presumably with small arrays of electrodes in each spinal segment, finer control of net torque with reduced charge density could be accomplished.

These types of studies will continue into the next quarter with position sensors being added to record precise limb position. It is anticipated that feedback of joint position will be necessary to accomplish smooth movement and joint stiffness since muscles fatigue, joint resistance, and a variety of non-linear elements are active during limb movement and positioning.

## III. External Urethral Sphincter (EUS) Neurons and Interneurons Determined by Pseudorabies Virus (PRV) Tracing Studies.

The purpose of the present study was to determine the location and distribution of neurons and interneurons which control the striated muscles of the EUS. The EUS is important in the storage and elimination of urine. Its activity (contraction to close the bladder outlet during storage and relaxation to open outlet during elimination) is coordinated with bladder activity to maintain continence and aid in elimination. The neurons which control the EUS and coordinate its activity with that of the bladder are located at various areas of the central nervous system including the lumbosacral spinal cord, brainstem and cortex. A transsynaptic tracer, PRV, was used in this study in order to identify not only first order motoneurons which project to the EUS muscle but also second and third order interneurons which are involved in EUS control at the spinal cord level. The methods used and the pathways involved in these studies are shown in Figure 4A and also briefly described in the following paragraph.

PRV (Becker strain) is injected bilaterally into the EUS of "pathogen free cats" under halothane:oxygen anesthesia. The animals are sacrificed 60 to 108 hours following PRV

injection. The spinal cord is removed, sectioned on a cryostat, processed with antibody for PRV and examined microscopically. PRV labeled neurons were observed in a variety of sites in the lumbosacral spinal cord (see Figure 5). In $S_1$ and rostral $S_2$ labeled neurons were found near the base of the ventral horn in an area known as Onuf's nucleus. Onuf's nucleus contains both large motoneurons and what appear to be smaller, possibly, interneurons. Interneurons were also seen in the dorsal commissure (DCM), sacral parasympathetic nucleus (SPN), and in the dorsal horn, especially in the superficial layers. Labeled neurons were also seen in the upper lumbar segment of the intermediolateral cell column (IML). This labeling probably represents sympathetic input to blood vessels of the EUS or to smooth muscles of the adjacent urethra. Supraspinal sites in the brainstem were also labeled in these experiments. The brainstem sites probably represent areas which coordinate bladder and EUS activity which are lost following damage to the spinal cord.

Since the Becker strain of the PRV labels neurons and interneurons via both the efferent and afferent pathways the $L_7$, $S_1$, $S_2$, and $S_3$ dorsal roots were cut bilaterally in two animals to eliminate labeled neurons via the afferent pathways. Figure 6 shows the distribution of labeled neurons following dorsal root section. Most of the dorsal horn labeling is gone while the labeled neurons in SPN, Onuf's, and DCM are still present, although somewhat reduced in the DCM.

These studies will provide sites for future microstimulation experiments which may be useful in modulating EUS activity and provide a framework for explaining the effects of microstimulation of areas of the spinal cord at sites remote from the first order efferent neurons. These studies will continue during the next quarter.

## IV. The Location and Distribution of Neurons and Interneurons which Control Prostate and Vas deferens.

The purpose of this study is to determine the location, in the spinal cord, of neurons which control the prostate gland and vas deferens. These neuronal sites would then be used as targets for microstimulation experiments to quantitate the ejaculatory responses in male cats.

Pseudorabies virus (PRV) was used in a similar manner as described above and shown schematically in Figure 4B. PRV was bilaterally injected into the prostate gland at the level of the vas deferens. Labeled neurons from these injections were found in the sacral ($S_1$, $S_2$, and $S_3$) and

lumbar ($L_2$, $L_3$, and $L_4$) spinal cord segments. The majority of labeled neurons were seen in $S_2$ and $L_3$ spinal cord segments with dense labeling in the sacral parasympathetic nucleus (SPN) and dorsal commissure (DCM) in the sacral segments and in the intermediolateral cell columns (IML) and to a lesser extent in the DCM in the lumbar cord. Some of the labeling in SPN and IML were preganglionic efferent neurons which provide the parasympathetic (from SPN) and sympathetic (from the lumbar IML) input to the prostate and vas deferens.

From information in the literature the major excitatory input to the smooth muscle of prostate and vas deferens is via the sympathetic nervous system in a variety of species, including the cat and primates. The parasympathetic inputs are thought to be involved in secretory function. Our PRV tracing studies together with physiological information suggest that microstimulation of sites in the lumbar spinal cord, especially in the IML and DCM of the $L_3$ cord, should produce ejaculatory responses consistent with what is suggested in the literature and our PRV tracing studies. The tracing studies will continue into the next quarter and microstimulation studies will begin during the next quarter with pressure recording from the vas deferens and prostate gland.

**Figure 1:** Schematic drawing of the experimental setup and methods used in these studies; showing the hindlimb of the cat to which a rotational torque sensor is attached via a bar pinned to the tibia. EMG electrodes are placed in the flexor and extensor muscles of the lower hindlimb (shank) which produce rotation about the knee joint. The EMG and torque sensor outputs are amplified and displayed on a chart recorder and recorded on tape. These signals are also digitized (2000 samples/sec.) and displayed and stored in a computer. Both on-line and post experiment analyses are provided by the computer and supporting software.
The spinal cord is exposed from the $S_3$ to $L_4$ spinal segments, and is stimulated at two different sites ($L_6$ and either $S_1$ or $L_7$) with fine tipped (300 - 400 $u^2$ exposed area) activated, iridium electrodes. The cord is stimulated at 200 u increments beginning at the cord surface and continuing to the base of the spinal cord. EMG and isometric torque are recorded at each depth. In these experiments each electrode is independently positioned.

**Figure 2:** Computer generated plots showing changes in flexion and extension torque (top trace each panel) and EMG from hindlimb extensor (middle trace each panel) and flexor (bottom trace each panel) muscles to microstimulation of the spinal cord at two sites $L_6$ and $S_1$. The top trace in each panel indicates flexion as an upward deflexion and extension as down. Each panel A to F is torque generated at a different intensity of stimulation of the extensor ($L_6$) and flexor spinal cord sites. The site in the spinal cord which produces flexion ($S_1$) is stimulated at an intensity of 100 uA in each panel. In panel A: $S_1$, the flexor site, is stimulated at 100 uA and the $L_6$ site, the extensor site at 0 uA. In B: $S_1$ = 100 uA, $L_6$ = 20 uA; C: $S_1$ = 100 uA, $L_6$ = 40 uA; D: $S_1$ = 100 uA, $L_6$ = 60 uA; E: $S_1$ = 100 uA, $L_6$ = 80 uA; F: $S_1$ = 100 uA, $L_6$ = 100 uA. Other stimulus parameters are 40 Hz, 0.2 msec, 12 seconds on and 120 seconds off. Notice that as the intensity of stimulation of the extensor is increased, flexor torque decreases (actually net torque decreases) until, in C, the amount of extensor torque just exceeds flexor torque. In C, a small net extensor torque is seen. Notice also the extensor EMG increases with increases in intensity of $L_6$ stimulation while the flexor EMG remains relatively constant.

**Figure 3:** A three dimensional surface plot for microstimulation of an extensor site in $L_6$ versus a flexor site in $L_7$ (A) and in $S_1$ (B). Both sites ($L_6$ and $L_7$ in A or $L_6$ and $S_1$ in B) are stimulated at seven intensities (0, 10, 20, 40, 60, 80, and 100 uA). To generate these plots all combinations of intensities for the two sites are tested and the net torque for each combination of intensities recorded. Other stimulus parameters remain constant throughout this study; they were 40 Hz, 0.2 msec pulse duration, 12 seconds on, 120 seconds off. At zero net torque the hindlimb should be held in a fixed position for all intensities that produce a zero net torque. Any deviation from zero net torque which would overcome knee joint resistance should move the shank in the direction of that net torque.

**Figure 4A:** A schematic diagram showing the experimental set-up and pathways involved in EUS tracing studies. The efferent and afferent innervation of the EUS is via the pudendal nerve. The urethra, which is surrounded by, and interdigitates with the striated muscle of the EUS, is innervated by postganglionic sacral parasympathetic fibers in the pelvic nerve and postganglionic sympathetic fibers in the hypogastric nerve and sympathetic chain. (The sympathetic innervation arises in the lumbar cord and is not shown in this figure.) Pseudorabies virus (PRV) is injected into external urethral sphincter muscle (EUS) bilaterally, labeling both first order neurons (via retrograde transport along the pudendal nerve) and interneurons by transsynaptic retrograde transport. Neurons at the supraspinal levels (brainstem, etc.) are also labeled by PRV, via long descending pathways. Since the smooth muscle of the urethra is adjacent to and interdigitated with the EUS striated muscle, afferents and efferents to the urethra are also labeled in our experiments.

**Figure 4B:** A schematic diagram showing the experimental set-up and pathways involved in the prostate gland and vas deferens tracing studies. The efferent and afferent (not shown) innervation of the prostate is via the hypogastric nerves (HGN) and pelvic nerves. The sympathetic innervation originates from preganglionic neurons in the IML of the lumbar spinal cord (only $L_3$ shown) and projects to the prostate via the HGN while the parasympathetic innervation originates from preganglionic neurons in the SPN and projects to the prostate via the pelvic nerves. PRV is injected bilaterally into the prostate at the level of the vas deferens labeling both neurons and interneurons in the pathway.

**Figure 5:** Camera lucida drawings of transverse sections of the spinal cord at four spinal levels ($S_1$, $S_2$, $S_3$, and $L_3$) showing the distribution of PRV labeled neurons at each level. Neurons from four sections are superimposed for each drawing. The dorsal roots were intact in this animal. Notice the labeled neurons at each level in the dorsal horn, Onuf's nucleus, DCM, SPN, IML, etc. Bar = 450 u.

**Figure 6:** Camera lucida drawings similar to Figure 5 except from an animal with dorsal roots sectioned bilaterally. Notice the reduction in density of labeled neurons, especially in the dorsal horn and DCM. Neurons from four sections are superimposed. Bar = 450 u.

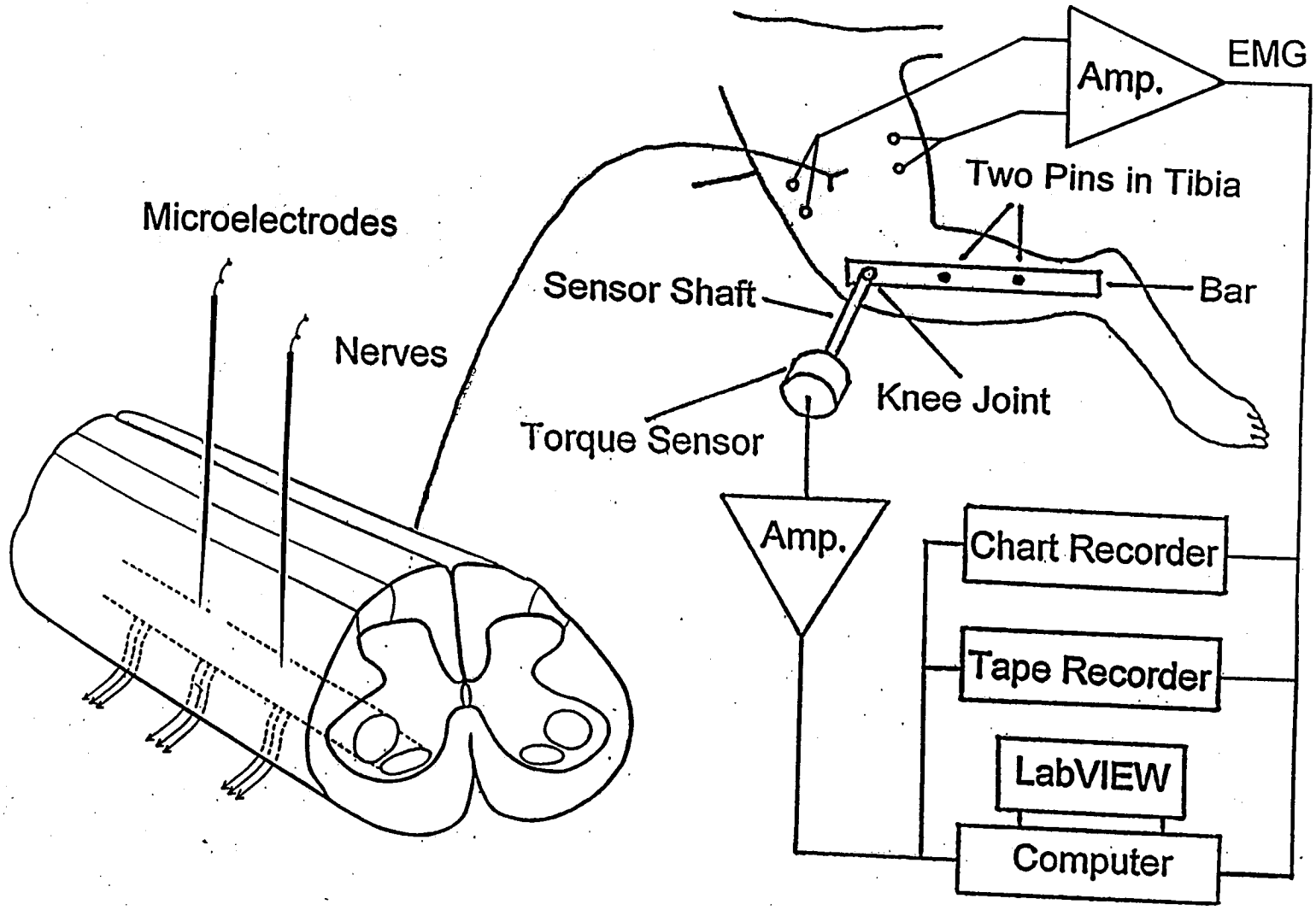

Amp.
EMG
Two Pins in Tibia
Microelectrodes
Sensor Shaft
Bar
Nerves
Knee Joint
Torque Sensor
Amp.
Chart Recorder
Tape Recorder
LabVIEW
Computer

Figure 1

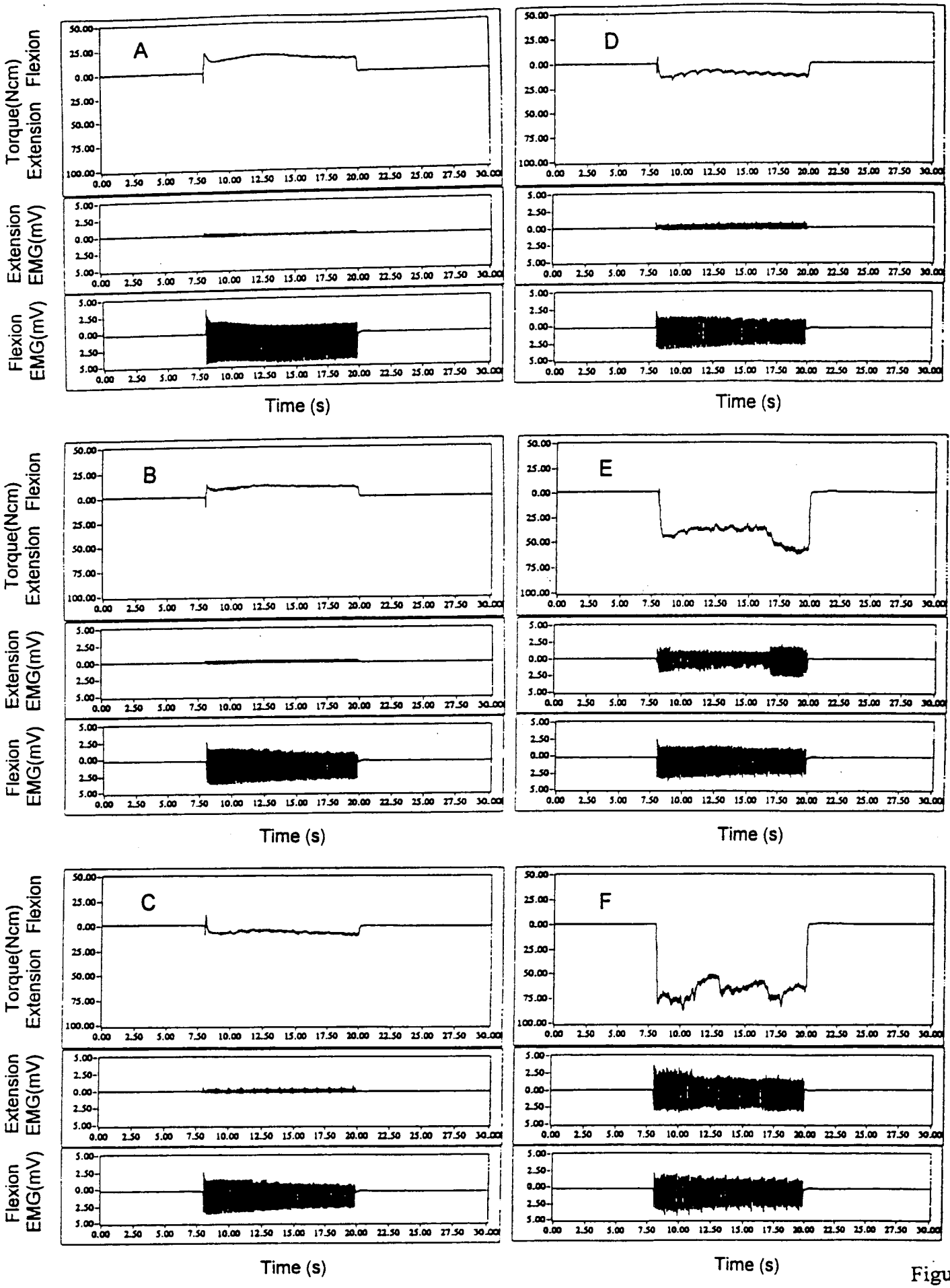
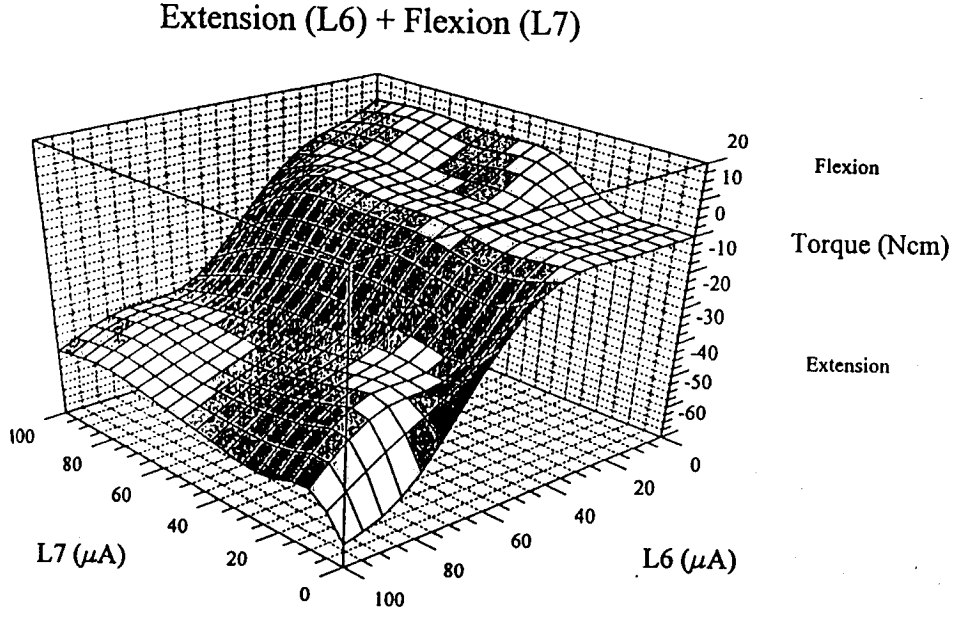

A
B
C
D
E
F
Torque(Ncm) Extension Flexion
Extension EMG(mV)
Flexion EMG(mV)
Time (s)

Figure 2

A.


Extension (L6) + Flexion (L7)
20
10
0
-10
-20
-30
-40
-50
-60
Flexion
Torque (Ncm)
Extension
100
80
60
40
20
0
L7 (μA)
0
20
40
60
80
100
L6 (μA)

B.

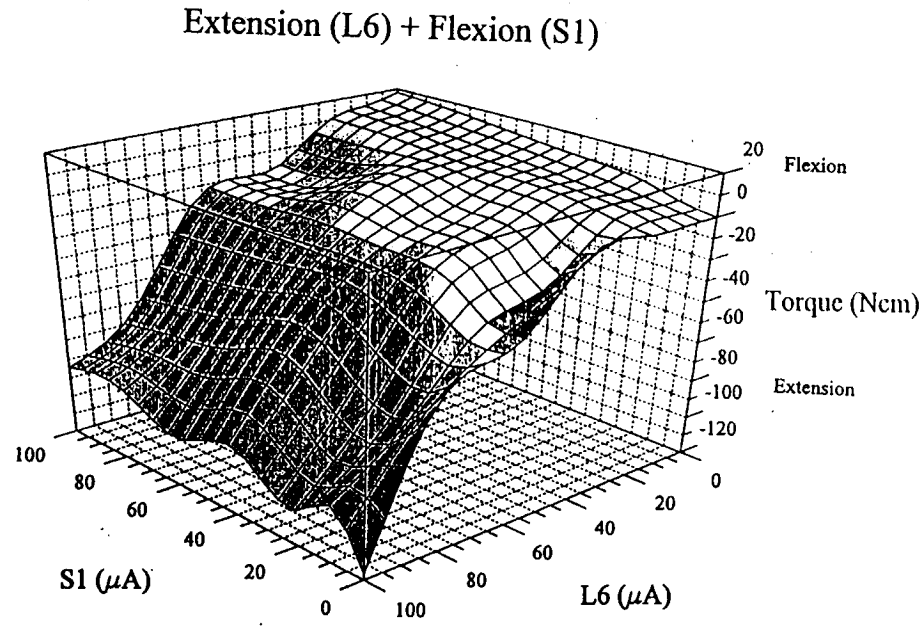
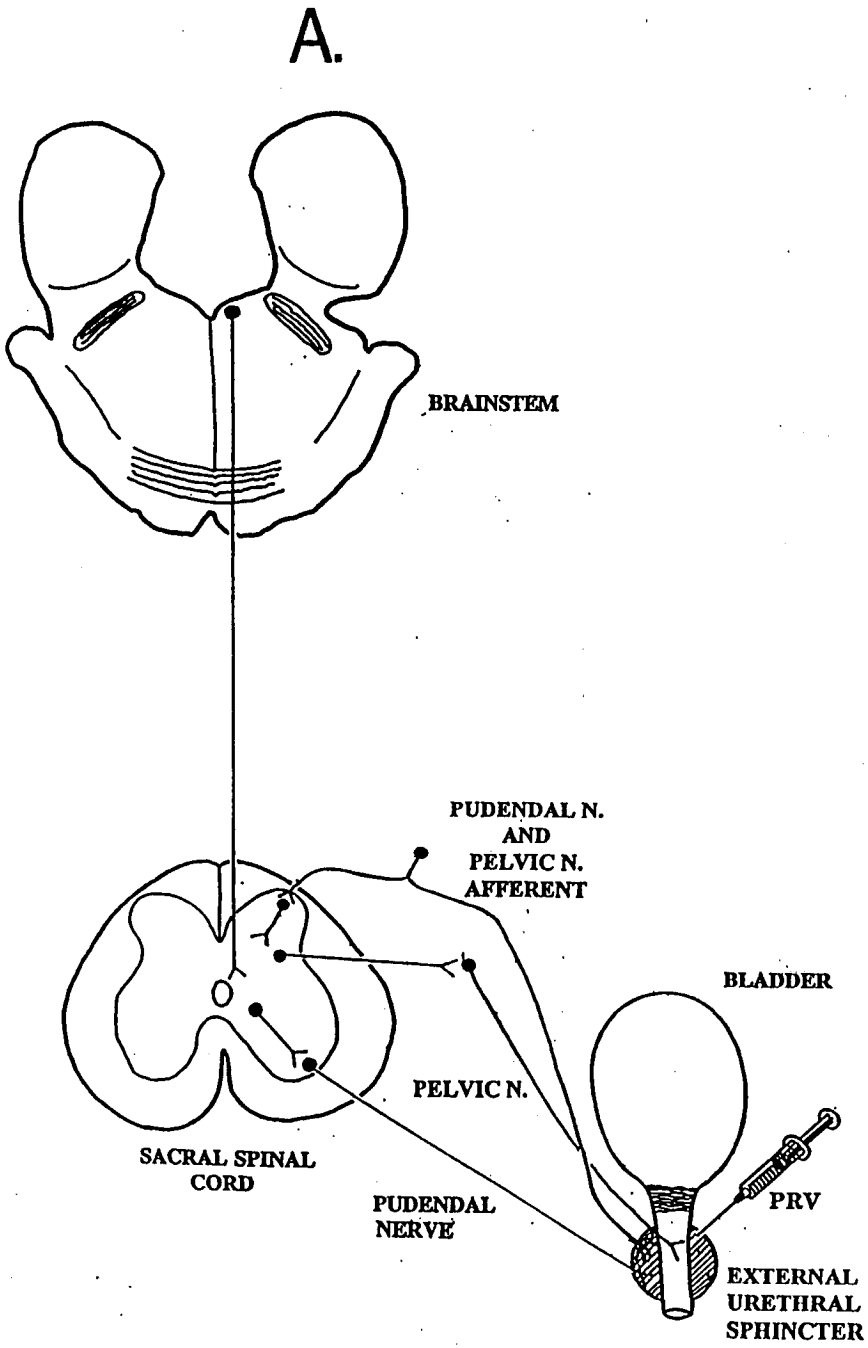

Extension (L6) + Flexion (S1)
20
0
-20
-40
-60
-80
-100
-120
Flexion
Torque (Ncm)
Extension
100
80
60
40
20
0
S1 (μA)
0
20
40
60
80
100
L6 (μA)

Figure 3


A.
BRAINSTEM
PUDENDAL N.
AND
PELVIC N.
AFFERENT
BLADDER
PELVIC N.
SACRAL SPINAL
CORD
PUDENDAL
NERVE
PRV
EXTERNAL
URETHRAL
SPHINCTER

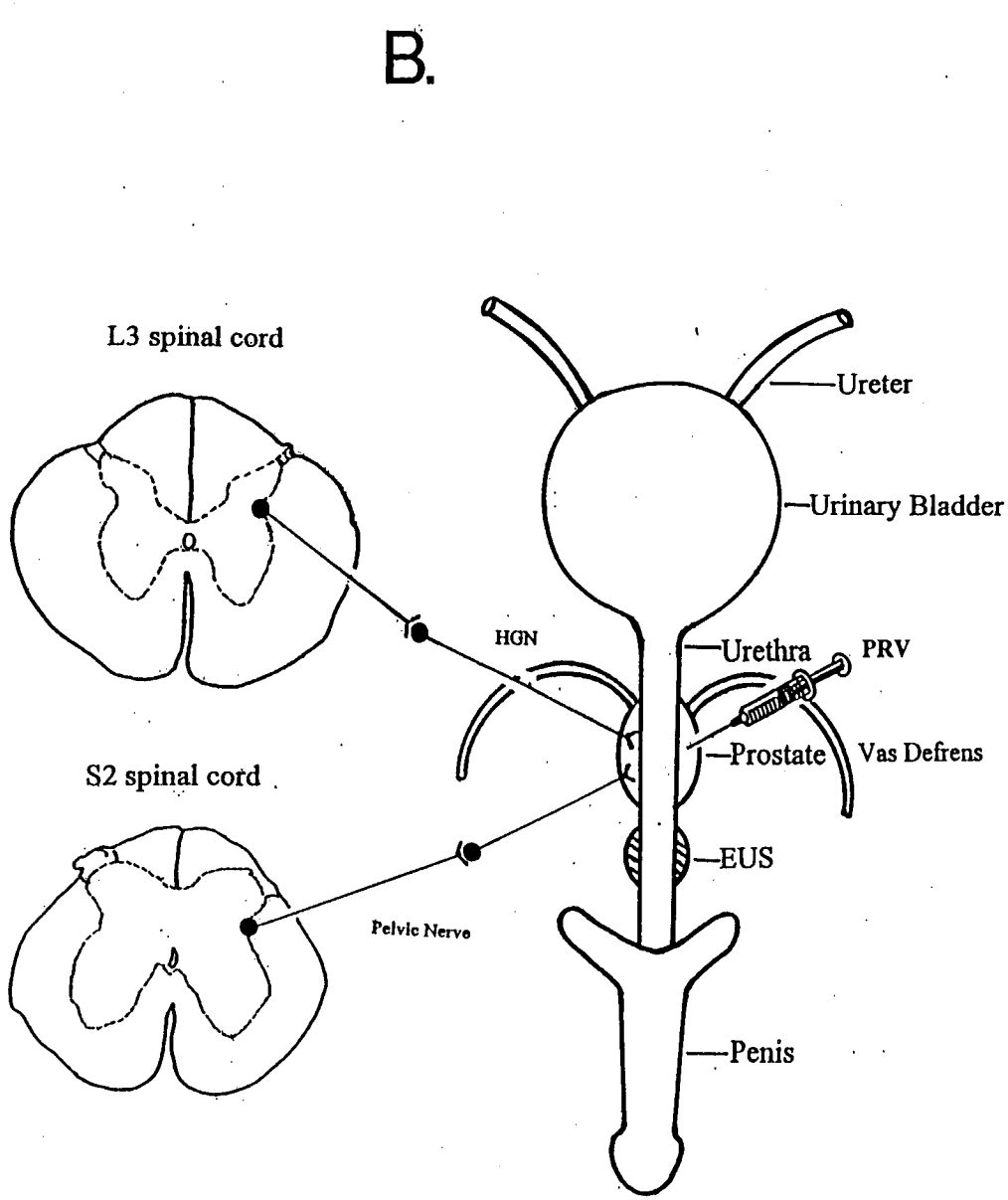
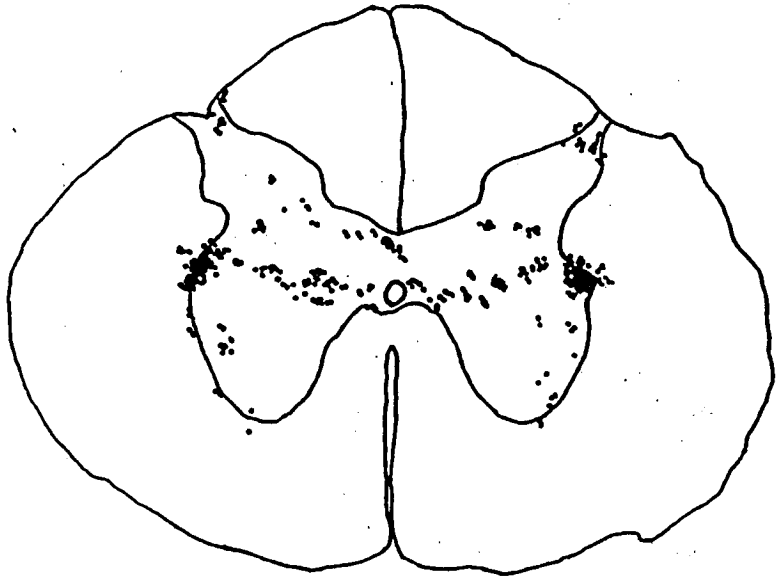
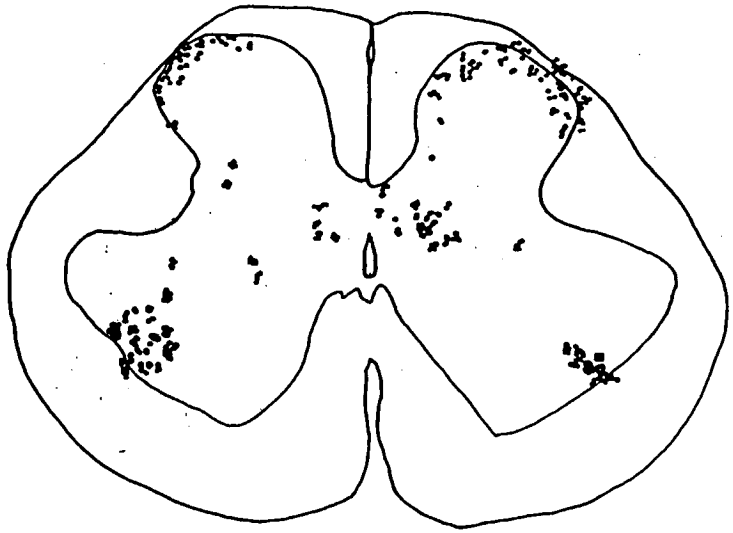

B.
L3 spinal cord
Ureter
Urinary Bladder
HGN
Urethra
PRV
Prostate
Vas Defrens
S2 spinal cord
EUS
Pelvic Nerve
Penis

Figure 4

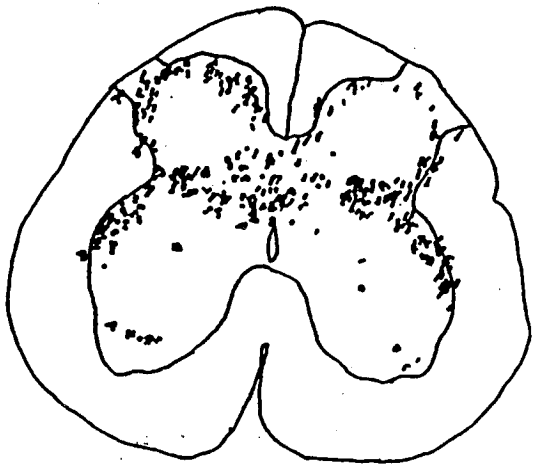
## EUS - Intact



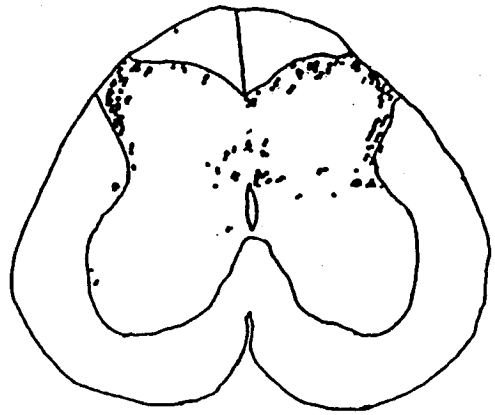
$L_3$



$S_1$



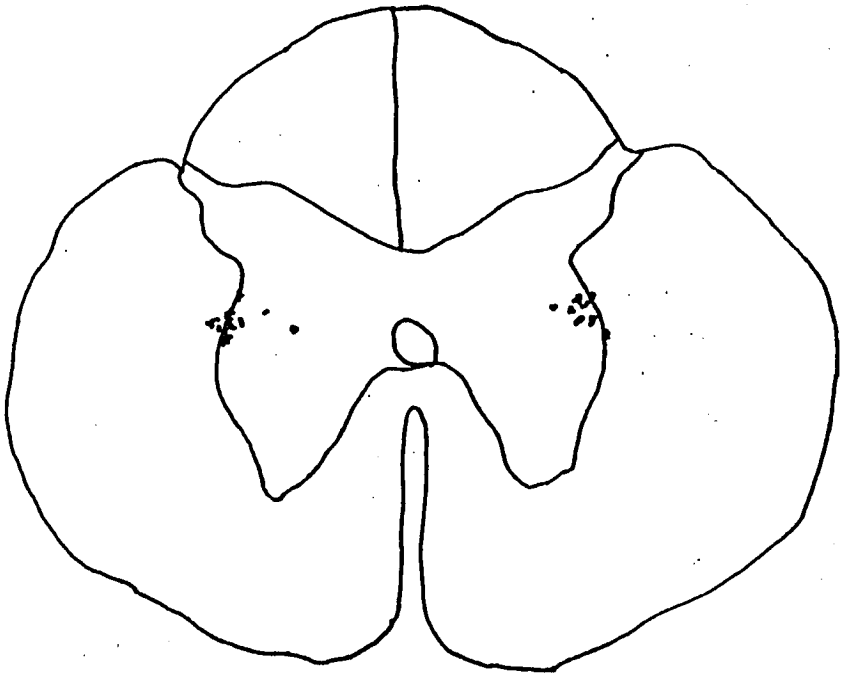
$S_2$



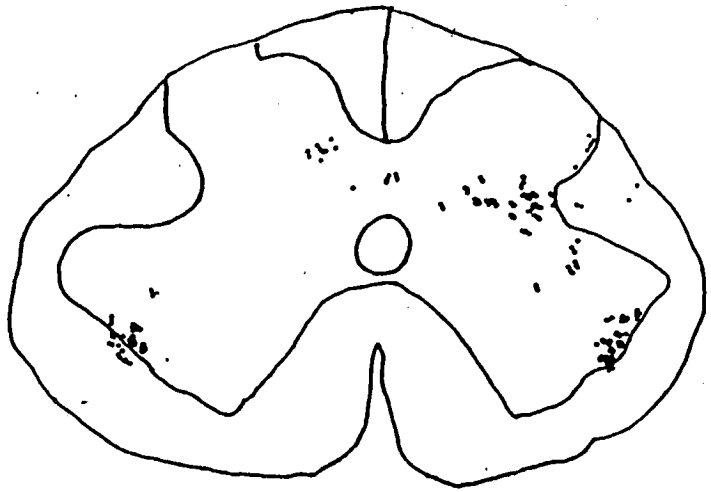
$S_3$

Figure 5

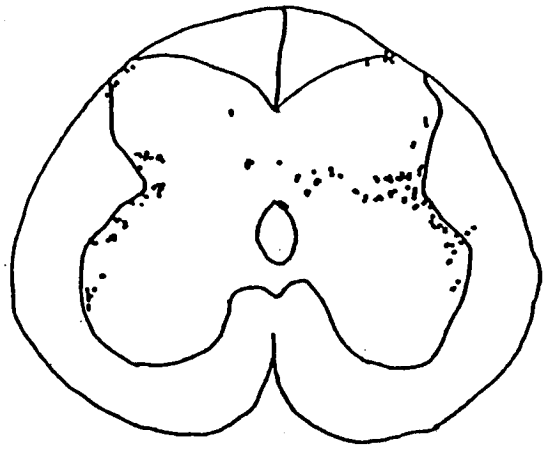
# EUS - Dorsal Root Cut



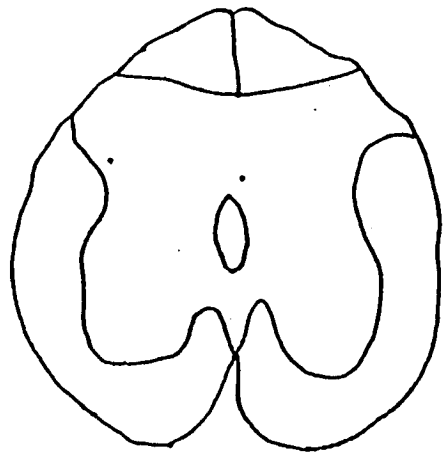
$L_3$



$S_1$



$S_2$



$S_3$

Figure 6